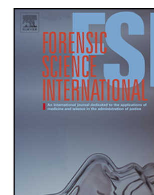
Forensic Science International 306 (2020) 110058

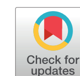
Contents lists available at ScienceDirect

Forensic Science International

journal homepage: www.elsevier.com/locate/forsciint



Simultaneous determination of synthetic cannabinoids and their metabolites in human hair using LC-MS/MS and application to human hair



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ARTICLE INFO

Article history:
Received 4 July 2019
Received in revised form 3 November 2019
Accepted 13 November 2019
Available online 16 November 2019

Keywords:
Synthetic cannabinoids
Hair analysis
LC–MS/MS

ABSTRACT

Hair is one of the key samples for judging drug abuse in the field of forensic science. However, few studies have examined synthetic cannabinoids and their metabolites in human hair. Synthetic cannabinoids are a class of chemicals that bind to cannabinoid receptors, but they differ structurally from the cannabinoids found in cannabis. They have been sold sprayed on dried, shredded plant material under brand names such as “Spice” since the 2000s. In South Korea, synthetic cannabinoids have been widely distributed since 2009 and many types detected up to now. Unlike traditional drugs such as methamphetamine and cannabis, the abuse trends of synthetic cannabinoids were variable by regions and changed according to the times. If new types of synthetic cannabinoids become popular which has been altered in some structures, it becomes difficult to identify using exist analytical method. Therefore, it is important to develop a new analytical method for synthetic cannabinoids currently being abused in society. In this study, we developed simultaneous analytical methods for the detection of 18 synthetic cannabinoids and 41 of their metabolites in authentic human hair samples using liquid chromatography-tandem mass spectrometry (LC–MS/MS). Selectivity, linearity, limits of detection (LODs), limits of quantification (LOQs), precision, accuracy, matrix effect, recovery, and process efficiency were evaluated, and all results were acceptable. Additionally, the distribution of synthetic cannabinoids in the head hair of Korean drug abusers from 2016 to 2018 was investigated. Hair samples from 43 individuals suspected of synthetic cannabinoid use were provided by law enforcement agencies. The drugs detected most prevalently in the head hair of Korean drug abusers were AB-CHMINACA and JWH-210.

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1. Introduction

Synthetic cannabinoids are a class of chemicals that bind to cannabinoid receptors but are structurally different from the cannabinoids found in cannabis [1,2]. Since 2004, herbal mixtures such as ‘Spice’ have been widely sold in many European nations and have gained popularity among young people [3–5]. These products are labeled to contain only herbal mixtures, but like cannabis, they induce psychoactive effects. By the end of 2008, synthetic additives such as CP-47,497 and JWH-018, which have a CB_1 (cannabinoid type 1) receptor binding affinity and psychoactive effects like Δ^9-tetrahydrocannabinol (THC), have been identified in these herbal mixtures [4,6]. Since then, a large number of new synthetic cannabinoids and ‘Spice’-like herbal products have continued to appear. Synthetic cannabinoids are mainly distributed in the form of herbal mixtures, but they are also in the form of tablets and powders. These products can contain one or more different types of synthetic cannabinoid components, and in the case of herbal products containing the same ingredient, large concentration differences have been measured [1,5]. Thus, there is a legitimate possibility of poisoning due to unintended excessive abuse. Synthetic cannabinoid abuse can cause anxiety, confusion, hypertension, psychosis, hallucinations, tachycardia, seizures and in severe cases, it can lead to death [7–11].

In South Korea, synthetic cannabinoids were first detected in 2009 and found to be widely distributed [12–15]. For this reason, South Korean government created a policy called the “temporary drug designation system” to control crime associated with the rapidly increasing use of synthetic cannabinoids [13,14]. Until 2010, JWH-018 and JWH-073 were the predominant forms of

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http://dx.doi.org/10.1016/j.forsciint.2019.110058
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cannabinoids found in synthetic cannabinoid cases. Since 2011, however, JWH-019, JWH-122, JWH-203, JWH-250, and AM-2201 have emerged on the Korean market [16]. Subsequently, halogenated analogues including XLR-11 and MAM-2201 have become increasingly prevalent [13]. In general, these halogenated cannabinoids have a higher affinity for the CB_1 receptor than non-halogenated forms [13,17]. AB-CHMINACA was first reported in Japan in 2013 and has since become distributed worldwide. AB-CHMINACA was introduced to South Korea in 2014 and quickly became the most abused synthetic cannabinoid until 2018 [14].

In the field of forensic science, hair is one of the most important samples for detecting drug abuse, including synthetic cannabinoids. The advantages of hair analysis include its longer detection window compared with blood and urine, its noninvasiveness, and its good sample stability [18,19]. Up to now, there have been some reports on the simultaneous determination of synthetic cannabinoids in human hair, but they were predominantly for parent drugs, not much their metabolites [20,21]. The purpose of this study was to establish and validate an analytical method for the simultaneous detection of 18 synthetic cannabinoid parents and 41 of their metabolites in human hair and apply this methodology to authentic human hair samples over the period of 3 years (2016–2018).

2. Materials and methods

2.1. Chemicals and reagents

AM-2201, AM-2201 N-(4-hydroxypentyl) metabolite (AM-2201 N-4-OH M), AM-2201 6-hydroxyindole metabolite (AM-2201 6-OH-indole M), JWH-018, JWH-018 N-(4-hydroxypentyl) metabolite (JWH-018 N-4-OH M), JWH-018 N-(5-hydroxypentyl) metabolite (JWH-018 N-5-OH M), JWH-018 N-pentanoic acid metabolite (JWH-018 N-COOH M), JWH-018 6-hydroxyindole metabolite (JWH-018 6-OH-indole M), JWH-019, JWH-019 N-(6-hydroxyhexyl) metabolite (JWH-019 N-6-OH M), JWH-073, JWH-073 N-(3-hydroxybutyl) metabolite (JWH-073 N-3-OH M), JWH-073 N-(4-hydroxybutyl) metabolite (JWH-073 N-4-OH M), JWH-073 N-butanoic acid metabolite (JWH-073 N-COOH M), JWH-073 6-hydroxyindole metabolite (JWH-073 6-OH-indole M), JWH-081, JWH-081 N-(5-hydroxypentyl) metabolite (JWH-081 N-5-OH M), JWH-122, JWH-122 N-(4-hydroxypentyl) metabolite (JWH-122 N-4-OH M), JWH-122 N-(5-hydroxypentyl) metabolite (JWH-122 N-5-OH M), JWH-203 N-(4-hydroxypentyl) metabolite (JWH-203 N-4-OH M), JWH-203 N-(5-hydroxypentyl) metabolite (JWH-203 N-5-OH M), JWH-210, JWH-210 N-(4-hydroxypentyl) metabolite (JWH-210 N-4-OH M), JWH-210 N-(5-hydroxypentyl) metabolite (JWH-210 N-5-OH M), JWH-250, JWH-250 N-(5-hydroxypentyl) metabolite (JWH-250 N-5-OH M), JWH-250 N-pentanoic acid metabolite (JWH-250 N-COOH M), JWH-398 N-(4-hydroxypentyl) metabolite (JWH-398 N-4-OH M), JWH-398 N-(5-hydroxypentyl) metabolite (JWH-398 N-5-OH M), MAM-2201, MAM-2201 N-(4-hydroxypentyl) metabolite (MAM-2201 N-4-OH M), MAM-2201 N-pentanoic acid metabolite (MAM-2201 N-COOH M), UR-144, UR-144 N-(5-hydroxypentyl) metabolite (UR-144 N-5-OH M), UR-144 N-pentanoic acid metabolite (UR-144 N-COOH M), UR-144 Degradant N-pentanoic acid metabolite (UR-144 degradant pentanoic acid), XLR-11, XLR-11 N-(4-hydroxypentyl) metabolite (XLR-11 N-4-OH M), AKB-48, AKB-48 N-(5-hydroxypentyl) metabolite (AKB-48 N-5-OH M), AKB-48 N-pentanoic acid metabolite (AKB-48 N-COOH M), 5F-AKB-48, 5F-AKB-48 N-(4-hydroxypentyl) metabolite (5F-AKB-48 N-4-OH M), AB-PINACA, AB-PINACA N-(4-hydroxypentyl) metabolite (AB-PINACA N-4-OH M), AB-PINACA pentanoic acid metabolite (AB-PINACA N-COOH M), 5F-AB-PINACA, 5-fluoro AB-PINACA N-(4-hydroxypentyl) metabolite (5F-AB-PINACA N-4-OH M), PB-22, PB-22 N-(4-hydroxypentyl) metabolite (PB-22 N-4-OH M), PB-22 N-(5-hydroxypentyl) metabolite (PB-22 N-5-OH M), PB-22 N-pentanoic acid metabolite (PB-22 N-COOH M), AB-CHMINACA, AB-CHMINACA metabolite M1A (AB-CHMINACA M1A), AB-CHMINACA metabolite M2 (AB-CHMINACA M2), AB-CHMINACA metabolite M4 (AB-CHMINACA M4), AB-FUBINACA, AB-FUBINACA metabolite 2A (AB-FUBINACA M2), JWH-018-d_9 and JWH-018 N-5-OH M-d_5 (JWH-018 N-(5-hydroxypentyl) metabolite-d_5) were purchased from Cayman Chemical (Ann Arbor, MI, USA).

HPLC grade methanol and acetonitrile were purchased from Fischer Scientific Co. (Fair Lawn, NJ, USA). Distilled water was prepared using a Milli-Q water purification system (Millipore, Bedford, MA, USA). All other chemicals were the highest quality available and used without further purification.

2.2. Sample preparation

Possible contaminants on the surface of the hair samples were eliminated by sequentially washing with methanol and distilled water using Agilent Bond Elut™ 3 mL reservoirs installed on a manifold. Hair samples (about 20 mg) were cut finely into 1 mm pieces with scissors and washed with methanol. The samples were then dried at room temperature. For the calibration and quality control (QC) samples, drug-free human hair was used which provided voluntarily. Prepared drug-free human hair was washed and cut finely into 1 mm and then dried at room temperature. After above procedures, about 20 mg of shredded hair was weighed and transferred to each reservoir and standard solution was added appropriately and dried at room temperature.

Analytes were extracted by incubating with 2 mL of methanol at 38 °C with continuous stirring for 20 h. JWH-018-d_9 and JWH-018 N-(5-hydroxypentyl) metabolite-d_5 were used as internal standards for the parent drugs and metabolites, respectively. The extracts were collected in glass tubes and evaporated to dryness under nitrogen gas at 45 °C. The residues were reconstituted in 100 μL of a 1:1 (v/v) mixture of methanol and mobile phase component A, and then filtered (0.22 μm, PVDF). Finally, 5 μL of samples were injected to a LC–MS/MS instrument with electrospray ionization in positive ion mode.

2.3. LC–MS/MS analysis

The HPLC system comprised an Agilent 1290 infinity UHPLC system consisting of a binary pump, degasser, thermo-stated autosampler, and column oven compartment (Agilent Technologies, Palo Alto, CA, USA). Chromatographic separation was performed on a Zorbax Eclipse Plus C18 RRHD column (1.8 μm, 2.1 mm × 100 mm i.d.; Agilent Technologies, CA, USA) with a Zorbax Eclipse Plus C18 guard column (1.8 μm, 2.1 mm × 5 mm, Agilent Technologies, CA, USA). The column oven temperature was maintained at 40 °C, and the auto-sampler temperature was set at 10 °C. The mobile phase comprised 2 mM ammonium formate/0.2% formic acid in distilled water (A) and 2 mM ammonium formate/0.2% formic acid in methanol (B). The mobile phase was delivered at 0.3 mL/min in gradient mode.

The analyte concentrations were quantified using an AB SCIEX 5500 QTRAP® mass spectrometer equipped with a Turbo Ion Spray to generate the positive ions $[M+H]^+$. The optimum source and gas conditions for ionization were found to be: ion source voltage, 5500 V; turbo gas temperature, 600 °C; curtain gas, 30 psi; collision gas, medium; gas 1 (nebulizing gas), 50 psi; and gas 2 (heater gas), 55 psi. Two multiple-reaction monitoring (MRM) transitions were selected for each analyte and data were processed using the Analyst® software. The MRM transitions, retention times, and other experimental parameter are shown in Supplementary Table S1.

2.4. Method validation

To validate the developed method, its specificity, selectivity, matrix effect, recovery, process efficiency, linearity, limit of detection (LOD), low limit of quantification (LLOQ), precision, and accuracy were examined. The method was validated according to SWGTOX guidelines [22] and Peters et al. [23] with few modifications. Specificity and selectivity were evaluated by analyzing ten different sources of drug-free human hair samples to demonstrate the absence of chromatographic alteration or interference from endogenous substances. To demonstrate linearity, analytes were spiked with blank human hair at known concentrations and analyzed using the optimized procedure. The LOD was determined as the lowest concentration tested in which the peak signal was three times higher than the chromatogram background. The LLOQ was determined as the lowest

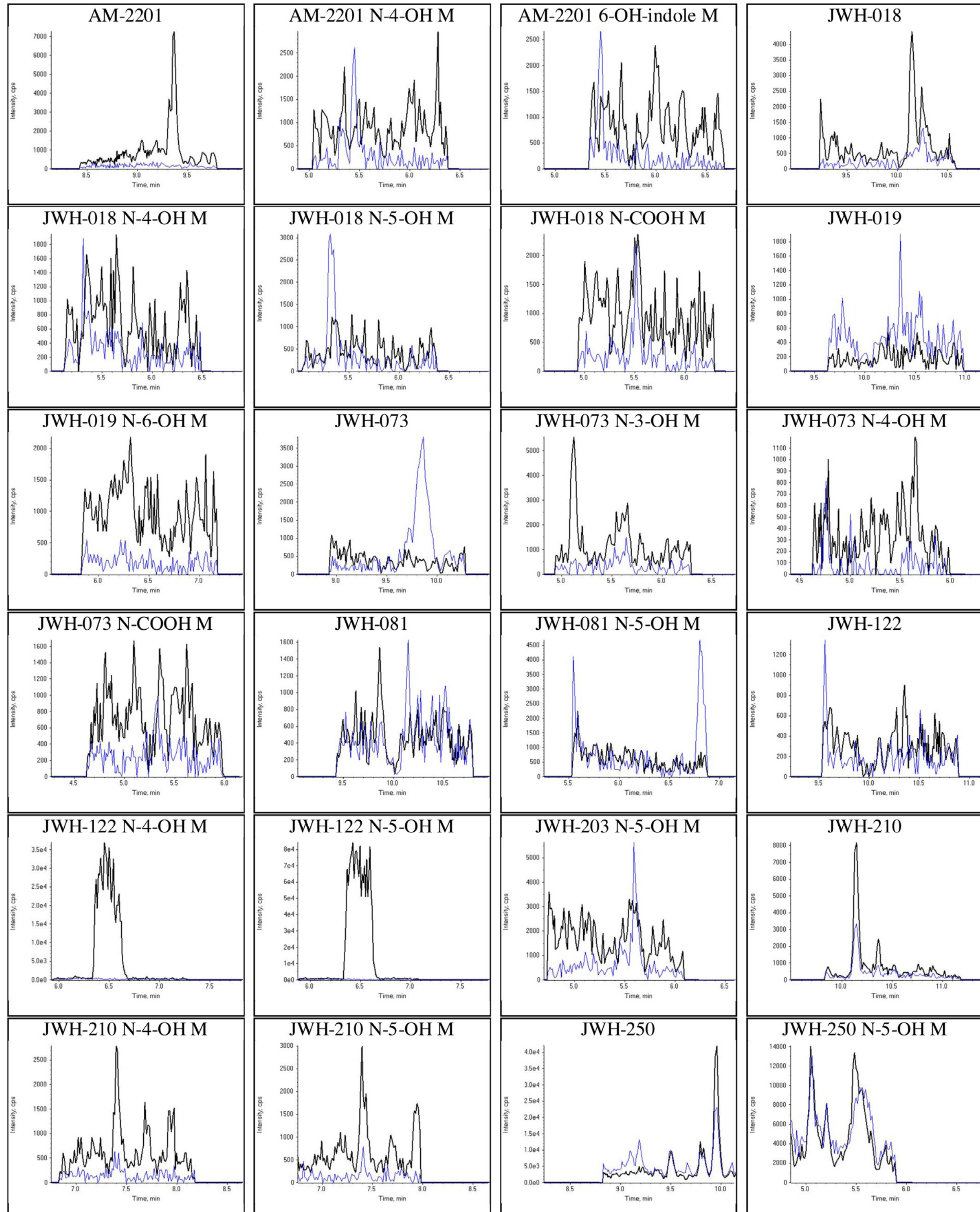

Fig. 1. Representative chromatograms of synthetic cannabinoids and their metabolites in authentic (case no. 2 in Table 3) hair samples.

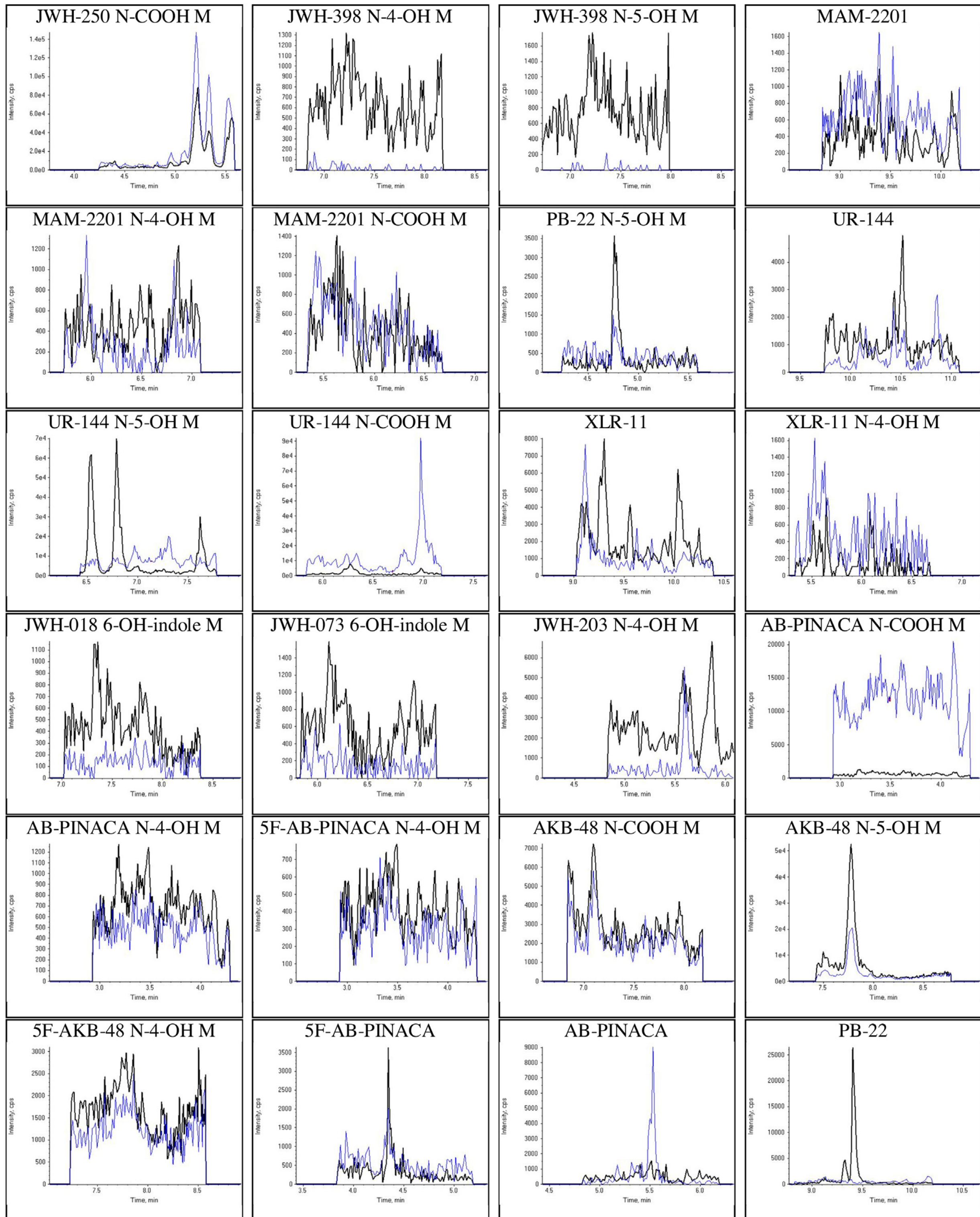

Fig. 1. (Continued)

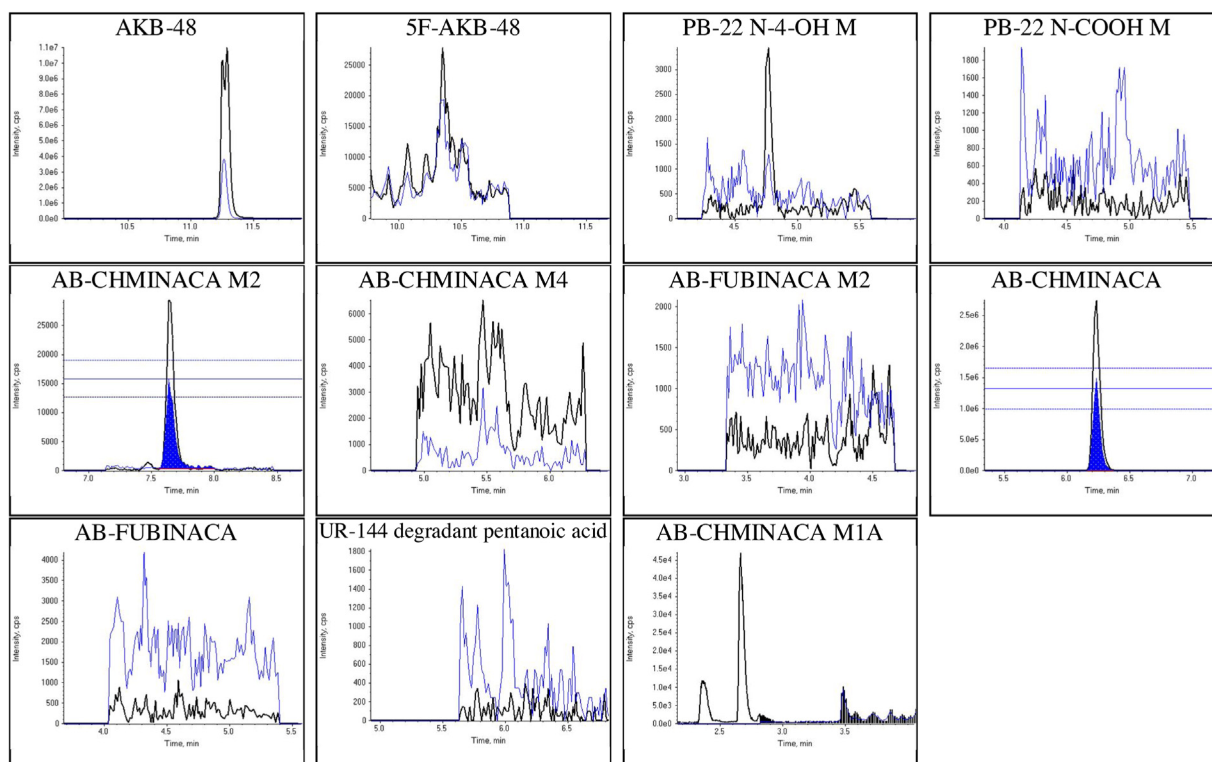

Fig. 1. (Continued)

concentration with less than ±20% coefficient of variation (CV) for precision and with less than ±20% bias accuracy. The intra-day (n = 5) and inter-day (five different days, n = 25) precision and accuracy were determined using the QC samples. For assessment of the auto-sampler stability, drug-spiked hair extracts at low and high concentrations (three samples per concentration level) measured at 0, 24, 48, and 72 h after storage at 10 °C in the auto-sampler.

The matrix effect, recovery, and process efficiency were evaluated by comparing the peak areas of analytes for three different sets: a neat standard set (set 1), extracts from five different sources of blank human hair spiked with standard after extraction (set 2), and extracts from five identical sources of blank human hair spiked with standard before extraction (set 3). The matrix effect was calculated as a percentage of the sample peak areas of set 2 relative to those of set 1. The recovery was calculated as a percentage of the sample peak areas of set 3 relative to those of set 2. Finally, the process efficiency was calculated as a percentage of the sample peak areas of set 3 relative to those of set 1 [23].

2.5. Quantitative analysis of authentic human hair samples

Hair samples from 43 individuals suspected of synthetic cannabinoid use were provided by law enforcement agencies between January 2016 and December 2018. All hair samples were prepared and analyzed as described above.

3. Results and discussion

3.1. Validation study in human hair

A quantitative LC–MS/MS method for simultaneous detection of 18 synthetic cannabinoids and 41 of their metabolites in human hair was developed and fully validated. Supplementary Figs. S1 and S2 show the representative chromatograms of analytes obtained from drug-free and drug-spiked (50 pg/mg) hair sample, respectively. Chromatographic separation was completed within 15 min and no peaks were detected for the blank hair sample (Fig. S1). There were no interfering peaks derived from endogenous substances at the elution times of the analytes (Fig. S2). The quantifier ion transitions were shown black signals and the qualifier ion transitions were shown blue signals, and integrated exact qualifier ion transitions were shown blue-painted peak. Fig. 1 shows chromatograms of hair segment from a subject with suspected abuse of synthetic cannabinoids (case no. 2 in Table 3) and detected AB-CHMINACA and AB-CHMINACA M2. To assess the selectivity of this method, ten different sources of blank hair were analyzed to verify the absence of chromatographic signals from interfering analytes. This method was selective and sensitive for the detection of synthetic cannabinoids and their metabolites in human hair.

The LOD, limit of quantification (LOQ), and linearity are summarized in Supplementary Table S2. Good linearity was achieved within the range of 1–1000 (1, 5, 20, 100, 200, 500, and 1000 pg/mg), 2–1000 (2, 5, 20, 100, 200, 500, and 1000 pg/mg), 5–1000 (5, 20, 50, 100, 200, 500, and 1000 pg/mg), 10–1000 (10, 20, 50, 100, 200, 500, and 1000 pg/mg), 10–10,000 (10, 50, 200, 500, 2000, 5000, and 10,000 pg/mg), 20–10000 (20, 50, 200, 500, 2000, 5000, and 10,000 pg/mg) or 20–20,000 (20, 100, 400, 1000, 4000, 10,000, and 20,000 pg/mg) pg/mg, depending on the analyte, and correlation coefficients (R) were larger than 0.99 for all analytes. The LODs ranged from 0.1–10 pg/mg hair and the LOQs ranged from 0.1–20 pg/mg hair. The LOQ values were defined as the lowest concentration with acceptable precision and accuracy (±20%, bias).

The intra- and inter-day precision and accuracy are shown in Supplementary Table S3. For all analytes, the developed method showed good precision and accuracy at low, medium, and high concentrations. From both the intra- and inter-day analysis, the precision CV% values were less than 11.5% and the accuracy (bias) ranged from -13.2–8.6% at low, medium, and high concentrations.

Table 1
Quantitative results of synthetic cannabinoids in authentic hair samples (2016 year).

No.	Gender	Age	Site	Hair length (cm)	Analytes	Concentrations (pg/mg)			
1	M	34	Scalp	7–10	Segment	0–10 cm	–	–	–
					AB-CHMINACA	228.1	–	–	–
					AB-CHMINACA M2	1.4	–	–	–
2	M	34	Scalp	10–15	Segment	0–12 cm	–	–	–
					AB-CHMINACA	851.4	–	–	–
					AB-CHMINACA M2	24.7	–	–	–
3	M	39	Scalp	7–15	Segment	0–3 cm	3–6 cm	6–9 cm	9–12 cm
					AB-CHMINACA	131.2	562.6	931.3	2184.3
					AB-CHMINACA M2	0.6	3.075	5.027	8.314
4	M	31	Scalp	4–5	Segment	0–5 cm	–	–	–
					AB-CHMINACA	185.4	–	–	–
					AB-CHMINACA M2	2.7	–	–	–
5	M	26	Scalp	5–12	Segment	0–12 cm	–	–	–
					AB-CHMINACA	3637.4	–	–	–
					AB-CHMINACA M2	75.9	–	–	–
					AB-CHMINACA M4	60.8	–	–	–
6	M	30	Scalp	5–8	Segment	0–8 cm	–	–	–
					AB-CHMINACA	524.0	–	–	–
					AB-CHMINACA M2	2.0	–	–	–
7	M	23	Scalp	5–13	Segment	0–12 cm	–	–	–
					AB-CHMINACA	8.9	–	–	–
					AB-CHMINACA M2	<LOQ	–	–	–
8	M	29	Scalp	10–12	Segment	0–12 cm	–	–	–
					AB-CHMINACA	1029.4	–	–	–
					AB-CHMINACA M2	12.1	–	–	–
9	M	35	Scalp	6–9	Segment	0–9 cm	–	–	–
					AB-CHMINACA	225.3	–	–	–
					AB-CHMINACA M2	4.8	–	–	–
10	M	27	Scalp	4–6	Segment	0–3 cm	3–6 cm	–	–
					AB-CHMINACA	285.8	133.961	–	–
					AB-CHMINACA M2	2.1	2.195	–	–
11	M	36	Scalp	7–10	Segment	0–10 cm	–	–	–
					AB-CHMINACA	51.7	–	–	–
					AB-CHMINACA M2	0.9	–	–	–
12	M	36	Scalp	5–11	Segment	0–3 cm	3–6 cm	6–11 cm	–
					AB-CHMINACA	ND	61.278	406.295	–
					AB-CHMINACA M2	ND	0.558	1.936	–
13	M	36	Scalp	8–12	Segment	0–12 cm	–	–	–
					AB-CHMINACA	22.5	–	–	–
					AB-CHMINACA M2	0.5	–	–	–
					JWH-210	0.1	–	–	–
					JWH-210 N-5-OH M	8.4	–	–	–
14	M	35	Scalp	2.5–8	Segment	0–8 cm	–	–	–
					AB-CHMINACA	1736.3	–	–	–
					AB-CHMINACA M2	28.5	–	–	–
15	M	34	Scalp	9–10	Segment	0–10 cm	–	–	–
					AB-CHMINACA	61.8	–	–	–
					AB-CHMINACA M2	3.2	–	–	–
16	M	26	Scalp	10–11.5	Segment	0–11.5 cm	–	–	–
					AB-CHMINACA	158.1	–	–	–
					AB-CHMINACA M2	5.3	–	–	–
17	M	34	Scalp	8–10	Segment	0–10 cm	–	–	–
					AB-CHMINACA	87.1	–	–	–
					AB-CHMINACA M2	2.0	–	–	–
18	M	28	Scalp	3–6	Segment	0–6 cm	–	–	–
					XLR-11	293.1	–	–	–
					UR-144 N-COOH M	2.6	–	–	–
19	M	35	Scalp	4–5	Segment	0–5 cm	–	–	–
					JWH-210	2.6	–	–	–
					JWH-210 N-4-OH M	147.4	–	–	–
					JWH-210 N-5-OH M	150.2	–	–	–
20	M	36	Scalp	8–11	Segment	0–11 cm	–	–	–
					JWH-210	0.1	–	–	–
					JWH-210 N-5-OH M	4.0	–	–	–
21	M	28	Scalp	6–7	Segment	0–7 cm	–	–	–
					PB-22	0.5	–	–	–
					PB-22 N-5-OH M	2.5	–	–	–
					AKB-48	0.2	–	–	–
					AKB-48 N-5-OH M	2.2	–	–	–
22	M	32	Scalp	6–8.5	Segment	0–8.5 cm	–	–	–
					5F-AKB-48	1169.5	–	–	–
					AKB-48	1.4	–	–	–
					AKB-48 N-5-OH M	1.7	–	–	–
					AKB-48 N-COOH M	<LOQ	–	–	–

Table 2
Quantitative results of synthetic cannabinoids in authentic hair samples (2017 year).

No.	Gender	Age	Site	Hair length (cm)	Analytes	Concentrations (pg/mg)			
1	F	44	Scalp	17–46	Segment	0–3 cm	3–6 cm	6–9 cm	9–12 cm
					AB-PINACA	ND	ND	2.491	12.437
2	M	42	Scalp	5–10	Segment	0–3 cm	3–6 cm	6–10 cm	–
					AB-FUBINACA	5.6	ND	24.375	–
3	M	37	Scalp	1.5–2.5	Segment	0–2.5 cm	–	–	–
					AB-CHMINACA	3.7	–	–	–
			Pubis	1–5.5	Segment	0–5.5 cm	–	–	–
					AB-CHMINACA	35.8	–	–	–
					AB-CHMINACA M2	2.6	–	–	–
4	F	33	Scalp	22–30	Segment	0–12 cm	–	–	–
					AB-CHMINACA	64.4	–	–	–
					AB-CHMINACA M2	0.8	–	–	–
5	F	35	Scalp	16–20	Segment	0–12 cm	–	–	–
					AB-CHMINACA	88.4	–	–	–
					AB-CHMINACA M2	1.4	–	–	–
6	M	29	Scalp	6–7.5	Segment	0–7.5 cm	–	–	–
					AB-CHMINACA	7.6	–	–	–
					AB-CHMINACA M2	1.5	–	–	–
7	M	27	Scalp	7–14	Segment	0–12 cm	–	–	–
					AB-CHMINACA	62.5	–	–	–
					AB-CHMINACA M2	1.4	–	–	–
8	M	32	Scalp	4–7	Segment	0–7 cm	–	–	–
					5F-AKB-48	1.2	–	–	–
					AKB-48 N-5-OH M	<LOQ	–	–	–
9	M	36	Scalp	3–14	Segment	0–12 cm	–	–	–
					AB-CHMINACA	485.3	–	–	–
					AB-CHMINACA M2	4.8	–	–	–
					JWH-210	4.9	–	–	–
					JWH-210 N-5-OH M	16.9	–	–	–
10	M	36	Scalp	6–10	Segment	0–3 cm	3–6 cm	6–10 cm	–
					AB-CHMINACA	280.8	5669.231	15,300	–
					AB-CHMINACA M2	1.0	7.677	31.8	–
					JWH-210	2.7	4.369	4.84	–
					JWH-210 N-5-OH M	18.2	68.769	106	–
11	M	32	Scalp	6–12	Segment	0–3 cm	3–6 cm	6–12 cm	–
					AB-CHMINACA	49.2	288.824	1650	–
					AB-CHMINACA M2	3.6	11.176	10.083	–
					JWH-210	1.5	1.953	2.738	–
					JWH-210 N-5-OH M	3.2	5.776	15.167	–
12	F	25	Scalp	8–12	Segment	0–3 cm	3–6 cm	6–12 cm	–
					AB-CHMINACA	71.0	112.222	644.545	–
					AB-CHMINACA M2	17.8	19.444	35.091	–
					JWH-210	<LOQ	<LOQ	<LOQ	–
					JWH-210 N-4-OH M	3.8	12.333	6.9	–
					JWH-210 N-5-OH M	4.3	12.111	7.164	–
13	M	29	Scalp	8–14	Segment	0–3 cm	3–6 cm	6–9 cm	9–12 cm
					AB-CHMINACA	60.0	316.471	495.294	2321.43
					AB-CHMINACA M2	12.1	5.759	10.647	19.643
					JWH-210	1.0	2.888	5.465	7.643
					JWH-210 N-4-OH M	5.2	16.882	17.471	27.643
					JWH-210 N-5-OH M	5.1	16.176	18.235	25.071
14	M	31	Scalp	5–11	Segment	0–3 cm	3–6 cm	6–11 cm	–
					JWH-210	<LOQ	0.137	0.868	–
					JWH-210 N-4-OH M	1.2	3.494	3.558	–
					JWH-210 N-5-OH M	1.2	3.194	3.337	–
15	M	33	Axillary	1–3	Segment	0–3 cm	–	–	–
					JWH-210	<LOQ	–	–	–
					JWH-210 N-4-OH M	3.1	–	–	–
					JWH-210 N-5-OH M	3.1	–	–	–
16	M	28	Scalp	6–8.5	Segment	0–8.5 cm	–	–	–
					XLR-11	1.8	–	–	–
17	M	35	Scalp	2–4.5	Segment	0–4.5 cm	–	–	–
					AB-CHMINACA	2.5	–	–	–
					AB-CHMINACA M2	1.0	–	–	–
18	M	34	Scalp	4–15	Segment	0–12 cm	–	–	–
					AB-CHMINACA	85.3	–	–	–
					AB-CHMINACA M2	9.2	–	–	–
					JWH-210	2.8	–	–	–
					JWH-210 N-4-OH M	<LOQ	–	–	–
					JWH-210 N-5-OH M	<LOQ	–	–	–

Table 3
Quantitative results of synthetic cannabinoids in authentic hair samples (2018 year).

No.	Gender	Age	Site	Hair length (cm)	Analytes	Concentrations (pg/mg)			
1	M	43	Scalp	6–9.5	Segment	0–3 cm	3–6 cm	6–9.5 cm	–
					AB-CHMINACA	8.2	33.9	72.5	–
					AB-CHMINACA M2	7.7	7.8	9.6	–
2	M	44	Scalp	12–17	Segment	0–12 cm	–	–	–
					AB-CHMINACA	241.6	–	–	–
					AB-CHMINACA M2	4.5	–	–	–
3	M	32	Scalp	3–12	Segment	0–12 cm	–	–	–
					AB-CHMINACA	117.2	–	–	–
					AB-CHMINACA M2	<LOQ	–	–	–
					AB-CHMINACA M1A	6.4	–	–	–
					JWH-210	4.1	–	–	–
					JWH-210 N-4-OH M	17.9	–	–	–
					JWH-210 N-5-OH M	12.1	–	–	–

Auto-sampler stability was assessed by measuring the processed samples in auto-sampler for 72 h. The auto-sampler stability results were within the acceptance criteria (±15%) compared to zero time analytes peak areas, showing that all analytes are highly stable at 10 °C.

The results of the matrix effect, recovery, and process efficiency of analytes in human hair are summarized in Supplementary Table S4. The mean matrix effect values ranged from 14.5–136.6% at the three concentrations examined, and the CV% values showed no significant variation for any of the analytes for different individuals. The matrix effect values of 5F-AKB-48 were ranged from 14.5–16.5 at the three concentrations and showed a highest ion suppression phenomenon. Contrary to this, AM-2201 6-hydroxyindole metabolite, 5F-AB-PINACA, AB-CHMINACA and AB-FUBINACA showed over 100% of matrix effect values, confirming the presence of ion enhancement. Recoveries and process efficiencies ranged from 70.9–114.4% and from 11.6–136.0%, respectively. For most analytes, there were no changes in the values of the matrix effect, recovery, and process efficiency as the concentration changed. In the case of the matrix effect and process efficiency, there were significant differences depending on the analytes, with the values for the metabolites higher than those for the parent drugs for most analytes.

3.2. Analysis of authentic hair samples

The validated method was applied to authentic hair samples from individuals suspected of synthetic cannabinoid use arrested by the police from January 2016 to December 2018. When the amount of hair was sufficient, it was divided into 3 cm length segments, otherwise, the entire hair was used for analysis. Hair samples were analyzed up to 12 cm of hair length. Synthetic cannabinoids were detected in 43 cases, and the quantitative results from this analysis are summarized in Table 1–3. As seen, AB-CHMINACA (32 cases), JWH-210 (12 cases), XLR-11 (2 cases), 5F-AKB-48 (2 cases), AKB-48 (1 case), PB-22 (1 case), AB-PINACA (1 case), AB-FUBINACA (1 case), and their metabolites were detected. The ages of the suspects ranged from 23 to 44 (mean = 33.1); 39 of them were male. AB-CHMINACA and JWH-210 were the most consistently detected synthetic cannabinoids in this study.

AB-CHMINACA was detected in 32 cases and 49 segments. In almost cases that AB-CHMINACA was detected, AB-CHMINACA metabolite M2 was also detected. Sim et al. [14] reported that AB-CHMINACA metabolite M2 was detected as the main metabolite in authentic hair samples of AB-CHMINACA abusers. The concentration ranges of AB-CHMINACA and AB-CHMINACA metabolite M2 were 2.5–15300.0 pg/mg (mean = 874.1) and 0.5–35.1 pg/mg (mean = 9.3), respectively. The concentrations of AB-CHMINACA and AB-CHMINACA metabolite M2 varied widely, and in all cases, the concentrations of the parent drug were found to be much higher than those of the metabolites. The concentration ratios of AB-CHMINACA to AB-CHMINACA metabolite M2 were highly variable, ranging from 1.06–738.5 (mean = 101.7). AB-CHMINACA metabolite M1A and M4 were detected in only 1 case and the concentration ratios of parent drug to metabolites were 18.3 and 59.8, respectively.

JWH-210 was detected in 12 cases and 23 segments. JWH-210 N-(4-hydroxypentyl) metabolite and JWH-210 N-(5-hydroxypentyl) metabolite were detected in 14 and 23 segments, respectively. JWH-210 N-(5-hydroxypentyl) metabolite was detected in all cases when JWH-210 was detected, but JWH-210 N-(4-hydroxypentyl) metabolite was detected in only 7 cases. The concentration range of JWH-210 was 0.06–7.6 pg/mg (mean = 2.8), and the concentrations of its two metabolites were higher than those of the parent drug. The values of the two JWH-210 metabolite concentrations were very similar and the concentration ratios of JWH-210 to the two JWH-210 metabolites ranged from 0.01 to 0.47 (mean = 0.2). In 8 cases and 17 segments, AB-CHMINACA and JWH-210 were detected together and the concentrations of AB-CHMINACA were higher than those of JWH-210.

XLR-11 and 5F-AKB-48 were detected in 2 cases and 2 segments, respectively. In one case, XLR-11 and UR-144 N-pentanoic acid metabolite were detected in an authentic hair sample from an individual suspected of XLR-11 use, whereas UR-144 was not detected at all. In this case, the concentration of XLR-11 was approximately 113-fold higher than that of UR-144 N-pentanoic acid metabolite. Wohlfarth et al. [24] reported that UR-144 N-pentanoic acid metabolite was derived from XLR-11 after oxidative defluorination, and Park et al. [13] reported that UR-144 N-(5-hydroxypentyl) metabolite and UR-144 N-pentanoic acid metabolite were detected as the main metabolites in authentic hair samples from individuals suspected of XLR-11 use. In the other XLR-11 case, XLR-11 metabolite was not detected at all. In this case, the detected XLR-11 concentration was very low. AKB-48 N-(5-hydroxypentyl) metabolite was detected as the main metabolite of 5F-AKB-48 in two cases. In one of these cases, 5F-AKB-48, AKB-48, AKB-48 N-(5-hydroxypentyl) metabolite and AKB-48 N-pentanoic acid metabolite were detected together. In this case, it is considered that 5F-AKB-48 was converted to AKB-48 after oxidative defluorination.

AB-PINACA and AB-FUBINACA were detected in 1 case and 2 segments, respectively, and their metabolites were not detected at all. AKB-48 and PB-22 were detected in 1 case and together in the same segment. AKB-48 N-(5-hydroxypentyl) metabolite and PB-22 N-(5-hydroxypentyl) metabolite were detected as metabolites, and the parent-to-metabolite concentration ratios were 0.09 and 0.2, respectively.

When compared the number of synthetic cannabinoids positive cases in authentic sample, we can see that it sharply decreased in

2018. It is more likely that the current trend of synthetic cannabinoids has changed rather than the abuse of that has decreased. Therefore, it is important to continue to investigate new synthetic cannabinoids analytical methods.

4. Conclusion

In this study, we developed simultaneous analytical methods for the detection of 18 synthetic cannabinoids and 41 of their metabolites in authentic human hair samples using liquid chromatography-tandem mass spectrometry (LC–MS/MS). The method was validated by selectivity, sensitivity, matrix effect, recovery, process efficiency, LOD, LOQ, linearity, accuracy and precision, and the parameters were in accordance with acceptable criteria. The validated method was successfully applied to determine the concentration levels of synthetic cannabinoids and its metabolites in authentic human hair samples.

Acknowledgement

This work was supported by National Forensic Service (NFS2017-2019, DNT-02), Ministry of the Interior and Safety, Republic of Korea.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.forsciint.2019.110058.

References

[1] I. Vardakou, C. Pistos, Ch. Spiliopoulou, Spice drugs as a new trend: mode of action, identification and legislation, Toxicol. Lett. 197 (2010) 157–162.
[2] R. Berkovitz, M. Arieli, E. Marom, Synthetic cannabinoids-the new “legal high” drugs, Harefuah 150 (884–887) (2011) 937.
[3] C. Mustata, M. Torrens, R. Pardo, C. Pérez, Psychonaut Web Mapping Group, M. Farré, Spice drugs: cannabinoids as a new designer drugs, Adicciones 21 (2009) 181–186.
[4] V. Auwärter, S. Dresen, W. Weinmann, M. Müller, M. Pütz, N. Ferreirós, ‘Spice’ and other herbal blends: harmless incense or cannabinoid designer drugs? J. Mass Spectrom. 44 (2009) 832–837.
[5] Susana Sadler Simões, Inês Silva, Antonio Castañera Ajenjo, M.ário João Dias, Validation and application of an UPLC–MS/MS method for the quantification of synthetic cannabinoids in urine samples and analysis of seized materials from the Portuguese market, Forensic Sci. Int. 243 (2014) 117–125.
[6] N. Uchiyama, R. Kikura-Hanajiri, N. Kawahara, Y. Haishima, Y. Goda, Identification of a cannabinoid analog as a new type of designer drug in a herbal product, Chem. Pharm. Bull. 57 (2009) 439–441.
[7] N. Schaefer, B. Peters, D. Bregel, et al., A fatal case involving several synthetic cannabinoids, Toxichem Krimtech 80 (2013) 248–251.
[8] A.L. Patton, K.C. Chimalakonda, C.L. Moran, K.R. McCain, A. Radominska-Pandya, L.P. James, C. Kokes, J.H. Moran, K2 toxicity: fatal case of psychiatric complications following AM2201 exposure, J. Forensic Sci. 58 (2013) 1676–1680.
[9] L.M. Labay, J.L. Caruso, T.P. Gilson, R.J. Phipps, L.D. Knight, N.P. Lemos, I.M. McIntyre, R. Stoppacher, L.M. Tormos, A.L. Wiens, E. Williams, B.K. Logan, Synthetic cannabinoid drug use as a cause or contributory cause of death, Forensic Sci. Int. 260 (2016) 31–39.
[10] P. Adamowicz, J. Gieroń, D. Gil, W. Lechowicz, A. Skulska, B. Tokarczyk, The effects of synthetic cannabinoid UR-144 on the human body-a review of 39 cases, Forensic Sci. Int. 273 (2017) e18–e21.
[11] A.B. Schneir, J. Cullen, B.T. Ly, “Spice” girls: synthetic cannabinoid intoxication, J. Emerg. Med. 40 (2011) 296–299.
[12] H. Chung, H. Choi, S. Heo, et al., Synthetic cannabinoids abused in South Korea: drug identifications by the National Forensic Service from 2009 to June 2013, Forensic Toxicol. 32 (2014) 82–88.
[13] M. Park, S. Yeon, J. Lee, S. In, Determination of XLR-11 and its metabolites in hair by liquid chromatography-tandem mass spectrometry, J. Pharm. Biomed. Anal. 114 (2015) 184–189.
[14] J. Sim, H.S. Cho, J. Lee, S. In, E. Kim, Determination of AB-CHMINACA and its metabolites in human hair and their deposition in hair of abusers, J. Pharm. Biomed. Anal. 140 (2017) 162–168.
[15] J. Kim, S. In, Y. Park, M. Park, E. Kim, S. Lee, Deposition of JWH-018, JWH-073 and their metabolites in hair and effect of hair pigmentation, Anal. Bioanal. Chem. 405 (2013) 9769–9778.
[16] M. Jang, W. Yang, I. Shin, H. Choi, H. Chang, E. Kim, Determination of AM-2201 metabolites in urine and comparison with JWH-018 abuse, Int. J. Legal Med. 128 (2014) 285–294.
[17] J. Nakajima, M. Takahashi, R. Nonaka, et al., Identification and quantitation of a benzoylindole (2-methoxyphenyl)(1-pentyl-1H-indol-3-yl)methanone and a naphthoylindole 1-(5-fluoropentyl-1H-indol-3-yl)-(naphthalene-1-yl)methanone (AM-2201) found in illegal products obtained via the Internet and their cannabimimetic effects evaluated by in vitro [35S]GTPγS binding assays, Forensic Toxicol. 29 (2011) 132–141.
[18] R. Wennig, Potential problems with the interpretation of hair analysis results, Forensic Sci. Int. 107 (2000) 5–12.
[19] F. Pragst, M.A. Balikova, State of the art in hair analysis for detection of drug and alcohol abuse, Clin. Chim. Acta 370 (2006) 17–49.
[20] M. Hutter, S. Kneisel, V. Auwärter, M.A. Neukamm, Determination of 22 synthetic cannabinoids in human hair by liquid chromatography-tandem mass spectrometry, J. Chromatogr. B 903 (2012) 95–101.
[21] F. Franz, H. Jechle, V. Angerer, M. Pegoro, V. Auwärter, M.A. Neukamm, Synthetic cannabinoids in hair - pragmatic approach for method updates, compound prevalences and concentration ranges in authentic hair samples, Anal. Chim. Acta 1006 (2018) 61–73.
[22] Scientific Working Group for Forensic Toxicology (SWGTOX), Standard practices for method validation in forensic toxicology, J. Anal. Toxicol. 37 (2013) 452–474.
[23] F.T. Peters, O.H. Drummer, F. Musshoff, Validation of new methods, Forensic Sci. Int. 165 (2-3) (2007) 216–224.
[24] A. Wohlfarth, S. Pang, M. Zhu, A.S. Gandhi, K.B. Scheidweiler, H.F. Liu, M.A. Huestis, First metabolic profile of XLR-11, a novel synthetic cannabinoid, obtained by using human hepatocytes and high-resolution mass spectrometry, Clin. Chem. 59 (11) (2013) 1638–1648.